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Issued December 21, 1910.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN NO. 74.
MILTON WHITNEY, Chief.

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CHEMICAL NATURE OF SOIL ORGANIC MATTER.

BY

OSWALD SCHREINER AND EDMUND C. SHOREY.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1910.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., July 23, 1910.

Sir: I have the honor to transmit herewith the manuscript of a scientific paper entitled "Chemical Nature of Soil Organic Matter," by Dr. Oswald Schreiner and Dr. Edmund C. Shorey, of this Bureau. This article is the result of an extensive study of a great variety of soils and reports the further isolation and identification of sixteen definite organic compounds from the soil, showing the great complexity of the organic matter and making clear its chemical nature and the processes of decay in the soil.

The investigation throws considerable additional light upon the question of soil fertility and I have, therefore, the honor to recommend that the article be published as Bulletin No. 74 of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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CHEMICAL NATURE OF SOIL ORGANIC MATTER.

INTRODUCTION.

Every soil investigator, whether it be the chemist, bacteriologist, or physicist studying some special problem, or the agronomist dealing with the general relation of soils to crops, sooner or later encounters difficulties that have their origin in the lack of knowledge of the chemical composition of the organic matter of the soil.

Some organic matter is essential to make a soil of what would otherwise be pulverized and more or less hydrolyzed rock, and while there are some soils capable of growing crops that contain very small quantities of organic matter, on the whole the quantity of this material in average soils is considerable. Analyses have shown that the average organic content of the soils of the United States is 2.06 per cent and of subsoils 0.83 per cent.[a]

This means 28 tons of organic matter per acre in the first 8 inches of soil and 50 tons in the soil and subsoil together to the depth of 2 feet.

The varied sources of this organic matter and the different classes of compounds that are known to result from the decomposition of this material have been fully discussed in a previous publication,[b] and it is necessary to state here simply the fact that this organic matter may have its source in the remains of any plant or animal, and the resulting compounds may be those that were in the living tissues and have resisted decay, those that result from a splitting or degradation of complex bodies present in the living plant or animal, or compounds arising through changes brought about by micro-organisms, and that nearly all classes of organic chemical compounds known may be represented.

Two views regarding the soil organic matter are at present current in agricultural literature. The first is some modification of the view of Mulder (1849),[c] "that at present seven different organic substances are known to exist in the soil. They are crenic acid, apocrenic acid, geic acid, humic acid and humin, ulmic acid and ulmin."

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[a] Cameron, Jour. Am. Chem. Soc., **27**, 256 (1905).

[b] Bul. 53, Bureau of Soils, U. S. Dept. Agr., p. 9 (1909).

[c] The Chemistry of Vegetable and Animal Physiology, trans. by Fromberg, 1849, p. 146.

This view, modified to the extent that some such body as humic acid, differing perhaps in different soils, but having the same general properties, makes up the humus and the greater portion of the organic matter of all soils is accepted by many agronomists and chemists. The acceptance of this view, which originated in the days when there was no organic chemistry in the present meaning of the term, can be attributed to two factors which have tended to retard the progress not only of agricultural chemistry but of other branches of science as well. These are the tendency to simplify or unify what proper thought would show to be complex; and second, the too prevalent habit of some investigators and most compilers of repeating or quoting scientific statements or deductions which have been made in the first place on wholly insufficient grounds. This view of soil organic matter has been fully discussed in a previous bulletin,[a] and it is not necessary to repeat that discussion here, for one of the results of the present paper is to show that the bodies that Mulder regarded as simple compounds related to each other are really made up of a large number of chemical compounds not necessarily related.

In strong contrast to this view of the simple composition of the soil organic matter is the second view current in agricultural literature, that the material is of a very complex nature, regarding which very little is known. Little fault can be found with this statement in itself, but it is often made in such a way as to convey the impression that not only do we know little regarding its complexity, but that it is almost hopeless to attempt any investigation of it. There is, moreover, seldom coupled with this confession of ignorance any appreciation of the importance of a thorough knowledge of the chemical composition of this important soil constitutent.

In considering the importance of the organic matter of the soil it should be borne in mind that it is material that is the result of change, and that much, perhaps all of it, is susceptible of still further change; that is, it is in a transition stage. The changes which it has undergone and the changes which it may still undergo are determined by a number of factors, chief of which are moisture, aeration, character of micro-organisms, and mutual relation of the organic compounds and the mineral constitutents. These factors are many of them influenced or controlled by the cultural methods, including fertilizing, drainage, irrigation, inoculation, etc., used in practical agriculture. While it is true that the soil, viewed from whatever point, presents dynamic problems, the study of the organic matter without doubt presents such problems in greatest complexity, but at the same time problems most susceptible of solution, once the character of the material is known.

[a] Bul. 53, Bureau of Soils, U. S. Dept. Agr., p. 21 (1909).

This view of the importance of soil organic matter concerns the agronomist and the farmer, but when the work of the special investigator is considered the need of definite knowledge is even more strongly emphasized. It is not necessary that the practical agriculturalist should know the chemical names or formulas of the organic compounds in the soil, but to the scientific investigator, to whom the farmer looks for the "why" of agricultural operations, such knowledge is necessary because it carries with it a knowledge of their properties. There can be intelligent chemical treatment of any material only when the chemical nature of the material treated is known. The treatment to which soil organic matter is subjected under cultural methods is in part at least chemical treatment in that such methods induce chemical changes. The operations of irrigation, conserving of moisture by mulches, aeration by cultivation, inoculation with cultures of bacteria, addition of organic and green manures, are all common agricultural methods used by farmers and they are also operations that influence the chemical changes which soil organic matter undergoes.

The influence of the organic matter of the soil may be considered under four heads: Its effect on the crop, its effect on the bacteria and fungi of the soil, its influence on the physical properties of the soil, and its relation chemically to the mineral ingredients of the soil.

It is a well-established fact that some chemical compounds which occur in plants and may get into the soil are harmful to growing plants when presented in water solution to the roots.[a] It has also been shown that some organic compounds that occur in soils and have been isolated from them are also harmful to growing plants under these conditions.[b] On the other hand, plants may take up other organic compounds when presented to their roots in water solution without injury to the plant, or in the case of some nitrogenous bodies, with benefit. Now, while the organic matter of soils is for the most part little soluble in water, a water extract of soils always contains some organic matter. In consequence organic compounds have always to be considered as a portion of the material in the nutrient solution supplied to crops growing in the soil.

The chief function of bacteria and fungi is to act on the higher organic compounds which make up living organisms and convert them into simpler compounds. In other words, these higher compounds are the food of the micro-organisms. The simpler compounds resulting from the activity of the fungi and bacteria commonly spoken of as the products of decay or fermentation, are, in

[a] Bul. 47, Bureau of Soils, U. S. Dept. Agr. (1907).
[b] Bul. 53, Bureau of Soils, U. S. Dept. Agr. (1909).

part at least, still organic substances and help to make up this portion of the soil. No fact regarding bacteria is better established than that they are influenced not only in habit of growth but also in the character of the compounds produced, by the chemical composition of the medium in which they are grown and are generally intolerant of the presence of an excess of their own by-products. The soil organic matter impregnated with the soil solution is then the culture medium in which soil micro-organisms have to grow and contains also the products of their growth. Bacteria, the activity of which is beneficial to crops, may fail to flourish because the food supplied them is not suitable or because their own products or the products of other forms hinder their growth. On the other hand, the activity of harmful bacteria, fungi, or protozoa may be stimulated by an abundant supply of suitable food. The necessity, then, of some chemical knowledge of this culture medium and by-products in any study of the mutual relation of soil micro-organisms to each other or to crops is apparent.

The properties of soils generally included under the term "physical," such as water-holding power, heat conductivity, absorption, and granulation, are universally recognized as potent factors in determining the character of a soil and its adaptability to the growing of crops. In considering these factors the tendency has been to consider the soil simply as an aggregate of mineral particles of different sizes, and consequently of different surface area, and to correlate the varying physical properties with this variation. That this view is wholly inadequate is evident, for solid organic matter may also be present in particles of different sizes, and these may have different physical properties due not only to variation in size, but probably in even greater degree to differences in chemical composition and structure. Furthermore, the organic matter may, and in fact generally does, play an intimate part in the behavior of the mineral particles, entering into chemical combination, coating them or cementing them together. The organic matter becomes, therefore, of the greatest importance in its influence on the great controlling factors in crop production, such as the solubility of the soil minerals, the physical structure of the soil granules, and the water-holding power of soils. To illustrate this, there was found in California a soil which could not be properly wetted, either by rain, irrigation, or movement of water from the subsoil, with the result that the land could not be used profitably for agriculture. On investigation it was found that this peculiarity of the soil was due to the organic matter, which when extracted had the properties of a varnish, repelling water to an extreme degree. The soil, once freed of this ingredient, had a high water-holding power. From other soils bodies of a waxy nature have been isolated, and it can thus be seen that

certain kinds of organic matter are important as the cause of the low water-holding power of some soils, although in general the presence of the organic remains of plants increases the power of soils to hold moisture—an important factor in crop production. It is evident, then, that there can be intelligent study of the influence that the organic matter of the soil has on its physical properties only when the chemical identity of its several components is known.

The great majority of organic chemical compounds are reactive toward inorganic compounds, acids, bases, and salts. Organic acids can form salts with mineral bases or double salts with mineral salts. Organic bases form salts with mineral acids, and quite a number of organic compounds combine both acid and basic properties and form organic compounds with both mineral acids and bases. Such being the case, there necessarily exists a mutual relation between the organic compounds in the soil and the mineral particles which form its foundation. Some of the acids isolated from the soils could not exist free for any time in a soil containing free bases or salts of weak acids, such as carbonic acid, and there is abundant evidence that many of the organic compounds exist in the soil in mineral combination. In fact, the relation between the organic and mineral particles of the soil is so intimate that any differentiation of soil chemistry into organic and inorganic should not be used as the basis of any theory or line of argument regarding soil phenomena or soil treatment.

In Bulletin 53 of this bureau the methods of isolating four organic compounds from soils was described and their properties given. The isolated compounds were: Dihydroxystearic acid, $C_{18}H_{36}O_4$; picoline carboxylic acid, $C_7H_7O_2N$; agroceric acid, $C_{21}H_{42}O_3$; and agrosterol, $C_{26}H_{44}O.H_2O$.

Since the publication in this bulletin of a review of the literature bearing on the nature of the organic matter of the soils, there have appeared several papers on the methods of humus determination and on the nature of the nitrogenous compounds resulting from the decomposition of the humus material with acids. None, however, have added any definite information regarding the identity of any compounds in the organic portion of the soil. A paper by Miklauz[a] is a valuable contribution to our knowledge of the variable character of humus material. This paper deals for the most part with the solubility of humus bodies in different solvents and the differences in the elementary composition of the separate fractions obtained by this means.

With the idea of the general importance of a greater knowledge of the chemistry of the soil organic matter in mind and with the view

[a] Beiträge zur Kentniss der Humussubstanzen. Zeit. f. Moorkultur und Torfverwertung, 285 (1908).

of furnishing more specific information of value in attempting to solve some of the problems presented by differences in or lack of fertility of soils, the work of which the present paper is a report was undertaken.

The work deals with a number of soils selected because of some problem presented by field observation, although these problems are not dealt with here. No one soil has been studied exhaustively, nor has there been any attempt to account for all of the organic matter in the soil in identified compounds. In some cases, however, this is approached by the sum of the identified compounds and separated fractions, the composition and properties of which are known but the identity of which has not as yet been established.

Two general methods have been used in the preliminary treatment of soils to obtain a solution from which to isolate organic compounds. These were the treatment with dilute alkali as in previous work and treatment with boiling 95 per cent alcohol. In several cases the same compounds were obtained by both methods.

It is quite evident that the compounds isolated from soils and described in this bulletin could not have been formed by any deep-seated change effected by the alkali or alcohol in the method of extraction. The alkali used was dilute, 2 per cent, the treatment was at room temperature and for a short time only, and the compounds isolated are not known to be formed by decomposition of more complex organic material by any such treatment. This treatment does effect changes in some of the organic matter of soils, but the material changed and the resulting compounds are not dealt with in this publication.

The after treatment of these solutions was also along two general lines. First, general treatments used in the separation or isolation of organic compounds, for the most part shaking out with immiscible solvents and precipitating with metallic salts, and, second, the direct search for certain compounds by methods already used for the isolation of such bodies in biochemical work.

When definite compounds were obtained by the general search, where the method used gave little or no clue to the identity of the compound obtained it was necessary to obtain sufficient of the body to determine its elementary composition and its general properties in order to establish its identity. When, however, a compound was obtained as the result of a direct search by an approved method, the correspondence of the properties, such as melting point, crystalline appearance, solubility, etc., of the compound obtained and the compound sought has been deemed sufficient to establish its identity.

The compounds obtained have been classified and described according to the group of chemical compounds to which they belong. In some cases compounds belonging to two or more groups have been

obtained as steps in one method, and if treated wholly from the point of view of method these would be described together. Since, however, the number of bodies isolated is constantly increasing, the classification according to method would soon result in confusion, and the chemical classification has been adhered to even when there was but one representative of a group. When compounds isolated have been obtained at different stages in the same method of treatment the fact is made clear in the text.

PARAFFIN HYDROCARBONS.

The paraffin hydrocarbons represent the simplest form of organic compounds and are widely distributed in nature. The lowest member of this group, methane or marsh gas (CH_4), is a constant product of the decomposition of organic matter under certain conditions. Higher members of the group, liquid and solid, occur as mixtures very widely distributed in the earth's crust as petroleum, asphalt, or similar deposits and also in mineral resins. The occurrence of paraffin hydrocarbons in plants is not very common, and so far as observed is confined to a few members of the group and to a few species of plants. The large deposits in the earth's crust can not, then, be attributed to the accumulation of unchanged plant residues, and a great many theories have been propounded to account for these deposits, most of them based on some transformation of organic material.

In spite of the fact that these hydrocarbons are of rather rare occurrence in plants and are seldom added to soil in plant débris, the wide distribution of extensive deposits of such compounds and the occurrence also of mineral resins and shales from which hydrocarbons can be obtained in the rocks from which soils are formed might lead one to expect to find some trace of these compounds in the soil. The question is also raised by this consideration whether some of the processes by which hydrocarbons have been formed from more complex organic compounds may not take place in the soil and escape recognition because the conditions are not favorable for the accumulation of these products in the soil. The production of the simplest paraffin hydrocarbon, methane, is known to take place in soil organic matter when submerged under water, as in swamps. The production here is supposed to be due to the activity of anaerobic micro-organisms, but it is not known that absolutely anaerobic conditions are essential to this production or that methane may not be formed in small quantities in ordinary soils. Methane is present in the gases from the combustion of fuel even when excess of oxygen is available, and it has been found in the combustion of organic compounds in elementary analysis that some of the carbon may be present in the products of combustion as methane instead of carbon dioxide, and

this when the combustion was carried on in pure oxygen.[a] It may be, therefore, that in the decomposition of organic matter the formation of methane rather than carbon dioxide depends on the structure or constitution of a portion of the material decomposed, and similarly more complex members of this series may also be formed.

In connection with this discussion of hydrocarbons in soils it should be noted that there are in nearly all soils dark-colored particles of organic matter that are very resistant to solvents and the action of either acids or alkalies. These sometimes are evidently particles of charcoal or coal that may have found their way into the soil, but a considerable portion is often made up of structureless bituminous-like material having the elementary composition so far as examined of lignites or brown coals. Whether this is formed in the soil is a matter for further investigation. Since such material often makes up a considerable portion of the organic content, its composition, constitution, and origin should be considered. One paraffin hydrocarbon has been isolated from a soil, and while in this particular case there is nothing to indicate that it may not be an unchanged plant residue, the isolation is of interest not only in accounting for a small portion of the organic matter, but also in that it opens up the question of a possible connection between the processes that go on in the soil and the processes by which paraffin hydrocarbons have been formed in nature.

HENTRIACONTANE, $C_{31}H_{64}$.

A peaty soil from North Carolina containing 27 per cent organic carbon when extracted with boiling 95 per cent alcohol yields a dark colored extract from which on cooling a light yellow micro-crystalline body separates. This material was separated by filtration and the alcohol removed from the filtrate by evaporation, the volume being kept constant by the addition of water. There was thus formed a reddish-brown precipitate which after separation and washing was in the form of a brown resinous powder. Extraction of this with boiling petroleum ether yielded a light colored solution, which after removal of the petroleum ether left a light yellow oily residue. This was saponified with alcoholic potash, the alcohol removed, and the soap dried and extracted with petroleum ether. On evaporation of the petroleum ether the residue was a light colored waxy mass, completely soluble in a relatively large volume of hot alcohol. On cooling the alcoholic solution so obtained, there was a separation of a feathery microcrystalline body which was collected, washed, and purified by recrystallizing several times from alcohol.

As thus obtained, the body was a hard, waxy mass, melting at 68° C., and having a specific gravity of 0.780 at its melting point. It

[a] Dunstan, Proc. Chem. Soc., **12**, 48 (1896).

was readily soluble in ether and petroleum ether, difficultly soluble in hot and very slightly soluble in cold alcohol. It was unacted on by fuming nitric acid at room temperature and did not absorb bromine.

Elementary analysis gave the following figures:

	Found.	Calculated for $C_{31}H_{64}$.
Carbon	84.14	85.32
Hydrogen	15.01	14.68

The method by which this body was obtained fixes it as either a hydrocarbon or a higher alcohol. Its behavior toward nitric acid and its elementary composition prove that it can not be a higher alcohol and its behavior toward bromine shows that it must be a saturated compound. It being thus fixed as a saturated or paraffin hydrocarbon, its melting point shows it to be hentriacontane.

Hentriacontane is a member of the paraffin series found in the paraffin residue of some petroleums. It has also been found in beeswax, together with another member of the same series, heptocosane, $C_{27}H_{56}$.[a] It occurs also in some plants, having been found in the leaves of *Gymnema silvestre*,[b] in tobacco leaves,[c] and in East India Ko-sam seeds (*Brucea sumatrana*).[d] With this information regarding its occurrence as a natural product and in the absence of any information regarding the possibility of its formation from other soil organic matter, this paraffin hydrocarbon may be regarded as an unchanged plant residue.

HYDROXY-FATTY ACIDS.

Hydroxy acids of the fatty series are very widely distributed in the vegetable kingdom. One or more of the three hydroxy acids—malic, citric, or tartaric—is usually present in an acid plant or fruit juice, and some of the less common, such as glycollic, also occur in plants. Some members of this group can be formed by micro-organisms from more complex material—lactic acid by the lactic fermentation of sugar and citric acid by citromycetes.[e] All members of the group, however, seem to be readily broken down by other bacteria or fungi into simpler compounds with the loss of the hydroxy character. Lactic acid goes readily to butyric and others to acetic or formic acid or formaldehyde. In view of this fact, while there is no doubt that

[a] Schwalb, Ann. Chem., **235**, 117 (1886).
[b] Power and Tutin, Pharm. Jour. (4), **19**, 234 (1904).
[c] Thorpe and Holmes, Jour. Chem. Soc., **79**, 982 (1901).
[d] Power and Lees, Pharm. Jour. (4), **17**, 183 (1903).
[e] Mazé, Ann. Inst. Pasteur, **23**, 830 (1909).

vegetable acids of this group are added to soils in large quantities and become temporarily, at least, a part of the organic matter of the soil, it does not seem likely that they would persist unchanged for any length of time.

Two hydroxy acids of the fatty series have been isolated from soils, but neither are known as natural products. These are α-hydroxystearic acid and the dihydroxystearic acid previously described.[a] Both must be looked on at present as the products of the action of micro-organisms, probably fungi, on some of the organic matter known to be of plant or animal origin.

While at present little is known of the processes by which these compounds are formed in the soil, and little can be said that is not of a speculative nature, it may be of interest at this point to note the laboratory methods by which hydroxy acids are formed. Three general methods are known by which the hydroxy acids are formed from the corresponding acid. These are: Making a halogen substitution product and treating this with silver oxide and water; making an amido derivative and treating this with nitrous acid; and making a sulphonic-acid derivative and treating it with caustic potash.

In attempting to find a parallel between these reaction and the processes likely to take place in the soil, the second method seems to be the only one that offers any foundation for a theory. It is known that amido compounds result from the decomposition of more complex nitrogenous compounds and may possibly be formed or built up by the action of ammonia which is commonly formed in soils. Nitrous acid, as the result of denitrification or as the first stage in the change of ammonia into nitrates, is formed in nearly all soils and would supply the reagent required in the second stage of the reaction.

In addition to these purely chemical methods of formation, there are the biological formations by fungi or bacteria to be considered, but this would lead somewhat afield from the purposes of the present bulletin.

α-MONOHYDROXYSTEARIC ACID, $C_{18}H_{36}O_3$.

When the humus extract of a soil obtained by extraction with dilute alkali is acidified, a brown or black flocculent precipitate of the so-called humus bodies is formed. If this precipitate is separated by filtration, washed, and treated while still moist with boiling 95 per cent alcohol, a portion of the precipitate goes into solution. The amount of the humus precipitate so dissolved varies with the character of the soil treated, but the alcoholic solution obtained in this way is always dark colored. On careful evaporation of this alcoholic solu-

[a] Bul. 53, Bureau of Soils, U. S. Dept. Agr. (1909). Jour. Am. Chem. Soc., **30**, 1599 (1908).

tion, adding water to keep the volume constant until the alcohol is removed, there is formed a brown or reddish-brown precipitate, which can be separated by filtration. When so separated, washed, and dried it is in the form of resinous lumps or powder, varying in color, melting point, and composition with the soil from which it was obtained.

On extracting this resinous material with petroleum ether there is obtained an extract generally colorless or light colored, which on evaporation of the petroleum ether leaves a residue which may vary from an oily, semisolid mass to a waxy solid. This residue is generally wholly soluble in hot alcohol. From this alcoholic solution there sometimes separates on cooling a white or yellowish microcrystalline mass or powder which in the case of one soil has been identified as α hydroxystearic acid.

The soil from which this compound was obtained was a sample of Elkton silt loam, a type covering a considerable area in Maryland. This soil is almost white in color, high in clay and silt, and contains 0.53 per cent organic carbon and 0.066 per cent nitrogen. The sample examined was one of several hundred pounds obtained from the Eastern Shore of Maryland.

This soil when treated with 2 per cent sodium hydroxide solution yields an extract much darker than might be expected from a soil so light in color. From this brown alkaline extract there is precipitated on acidifying with sulphuric acid a brown, flocculent mass. On separation of this by filtration, washing and treating with boiling 95 per cent alcohol, there is obtained an alcoholic solution nearly as dark in color as the original alkaline extract. The precipitate formed by evaporation of the alcohol and addition of water was, after filtration, washing, and drying, a brown mass easily pulverized.

Extraction of this brown powder with petroleum ether and evaporation of the solvent left an oily, semisolid, light yellow mass, which was completely soluble in hot 95 per cent alcohol. On cooling this alcoholic solution there separates a yellow, microcrystalline powder, which after separation by filtration, washing with cold alcohol, dissolving again in hot alcohol, and repeating the separation several times, can be obtained free of color and of constant melting point.

The substance so obtained melts at 84–85° C., is soluble with difficulty in cold alcohol, readily in hot alcohol, cold ether, or petroleum ether. It crystallizes usually in small irregular leaflets, but can be crystallized from alcohol by very slow cooling, in six-sided plates. It forms salts with alkalies soluble in water and can be obtained from such solution unchanged by acidifying and extracting with ether.

Elementary analysis of this compound gave the following figures:

	Found.	Calculated for $C_{18}H_{36}O_3$.
Carbon	72.09	72.00
Hydrogen	12.17	12.00
Oxygen	15.84	16.00

This composition corresponds with that of the monohydroxystearic acids, two of which are known, designated α and β, respectively, and having the formula $C_{18}H_{36}O_3$. The properties of the acid isolated from the soil fix it as the α acid. The α acid is much less soluble in alcohol than the β acid, and the β acid, moreover, forms an anhydride on heating with hydrochloric acid, which is not the case with the α acid nor the acid from the soil. The melting points of the α and β acids are so nearly the same that this property can not be used to distinguish between them. The melting point of the pure α acid, artificially prepared, remained unchanged on mixing with the acid from the soil, and this fact, together with the composition found and sparing solubility in alcohol and failure to form an anhydride, is sufficient to establish the identity of the acid from the soil as α-monohydroxystearic acid.

α-Hydroxystearic acid is easily made in the manner described by Sadomsky.[a] When stearic acid is treated with red phosphorus and bromine, α-bromostearic acid is formed. On heating this with alcoholic potash the potassium salt of α-hydroxystearic acid is formed, and on acidifying the aqueous solution of this salt the acid is set free and can be extracted with ether.

α-Hydroxystearic acid is not known to occur in any natural animal or vegetable product and up to the present has been isolated from only one soil.

The possible origin of this acid has already been discussed from a speculative point of view and while it is not known as a natural product it must be remembered that natural fats and oils are usually complex mixtures of glycerides and that the chemistry of such glycerides as occur in these products in small amounts is very incomplete. More complete chemical knowledge of the natural fats and oils may show the presence of α-monohydroxystearic acid. The method of its laboratory preparation does not suggest any possible parallelism in the soil as was the case when the origin of dihydroxystearic acid was under discussion.

In the earlier literature of the hydroxystearic acids there is some confusion of nomenclature, as well as disagreement regarding their constitution. The β acid described by Saytzeff [b] was designated as

[a] Ber. chem. Ges., **24**, 2388 (1891).

[b] Jour. prakt. Chem. (2), **35**, 369 (1887).

the α acid by Geitel.[a] The designations α and β acid seem to have been used primarily to distinguish isomers, the constitution of which were not known. The constitution, as now accepted and established by Shukoff and Schestakoff[b] is not in harmony with the common meaning of α and β designations. In the simple hydroxy-fatty acids the term α is applied to that acid having the hydroxyl group next the carboxyl group, for example, α-hydroxypropionic acid (lactic) is represented thus: $CH_3.CHOH.COOH$; the term β is applied to that acid having the hydroxyl on the second carbon atom, for example, β-hydroxypropionic (hydracrylic) is represented thus: $CH_2OH.CH_2COOH$. The constitution of the α and β hydroxystearic acids is represented as follows: α acid, $CH_3(CH_2)_6CHOH(CH_2)_9COOH$; β acid, $CH_3(CH_2)_7CHOH(CH_2)_8COOH$. In neither case is there α or β structure. The authors in discussing these formulas used the designations: 1. 11. for the α acid and 1. 10. for the β acid, 1 being the carbon atom of the carboxyl group and 10 and 11 the carbon atoms counting from 1 to which the hydroxyl group was attached. Since, however, these acids are still generally described and referred to in the literature as α and β these terms have been used in this paper with this explanation.

ACIDS OF UNKNOWN CONSTITUTION.

There are a great many chemical compounds, especially of natural origin, to which, while the group to which they belong is well established and their general properties are known, no structure or constitution has been assigned. This is simply another way of stating that all the properties are not known. This meager knowledge regarding constitution is marked among the higher fatty acids, especially those of infrequent occurrence.

Two acids of this character have been isolated from soils. These acids are isomeric, having the same elementary composition, and while one can be regarded as a well-established chemical compound, there is still some doubt whether the other is a single body or a mixture.

PARAFFINIC ACID, $C_{24}H_{48}O_2$.

The cold alcoholic solution from which α-hydroxystearic acid has separated in the manner just described is yellow in color and evaporation of the alcohol leaves a semisolid, oily mass. On standing some time crystals form in this mass, but the nature of the material was such that they could not be separated without great loss, and so this was not attempted. The oily mass was dissolved again in alcohol and an alcoholic solution of lead acetate added. This gave a yellow precipitate which was separated by filtration, washed with alcohol, suspended

[a] Jour. prakt. Chem. (2), 37, 53 (1888).

[b] Jour. prakt. Chem. (2), 67, 415 (1903).

in alcohol, and treated with hydrogen sulphide. After separation from the lead sulphide the alcoholic solution was allowed to evaporate slowly, leaving a somewhat waxy mass of leaflets. This mass was purified by dissolving again in alcohol and repeating the operation of precipitation with lead acetate and finally drying on a porous plate. The body so obtained was light yellow in color and melted at 45–48° C. Elementary analysis gave the following figures:

	Found.	Calculated for $C_{24}H_{48}O_2$.
Carbon	78.40	78.20
Hydrogen	13.29	13.00
Oxygen	8.31	8.80

This composition, the melting point, and physical properties correspond with paraffinic acid, $C_{24}H_{48}O_2$, described by Pouchet[a] as obtained by the action of fuming nitric acid on paraffin.

The research of Pouchet on which alone the existence of paraffinic acid rests is, as published, inconclusive as to this body being a definite chemical compound. The quantity of this substance obtained from the soil was insufficient for any extended research, and so far the character of the compound obtained from paraffin has not been studied except that the following facts were established. Paraffin was treated with fuming nitric acid in the manner described by Pouchet and a compound obtained having the properties and composition stated by him. Elementary analysis of this substance gave the following figures:

	Found.	Calculated for $C_{24}H_{48}O_2$.
Carbon	78.1	78.2
Hydrogen	13.3	13.0
Oxygen	8.6	8.8

The properties and composition of this compound corresponded with those of the substance obtained from the soil and there is little doubt that they are identical. With this understanding the name paraffinic acid is applied to the soil compound. This body has so far been obtained from but one soil, the Elkton silt loam already described. A number of soils have been examined that gave, at the point where paraffinic acid was obtained in the case of the Elkton silt loam, a precipitate with alcoholic lead acetate, but the quantity of material has been either too small for identification or has had other properties and composition.

[a] Bul. Soc. Chim., 23, 111 (1875).

Very little can be said regarding the possible origin of paraffinic acid in the soil. Several solid hydrocarbons of the paraffin series have been shown to occur in plants and may therefore be a part of the organic matter added to the soil. Oxidation of these in the soil might give rise to the body found.

It may be noted in this connection that it is well established that paraffin can be oxidized in the laboratory, but usually by rather vigorous treatment. Gill and Mensel[a] found that on treating paraffin with dilute nitric acid or chromic acid it was oxidized to cerotic acid, $C_{27}H_{54}O_2$, acetic acid, and succinic acid. Pouchet in the research referred to found succinic acid in addition to paraffinic acid, but no cerotic acid.

LIGNOCERIC ACID, $C_{24}H_{48}O_2$.

When a soil high in organic matter is carefully heated in a closed tube, there is obtained a dark-colored, tarry distillate, which in some cases sets on cooling to a semisolid, crystalline mass.

A peat soil containing 27 per cent organic carbon treated in this way gave a distillate of this character in considerable quantity. The semisolid mass on washing with cold alcohol was freed from much of its color with little loss of solid matter. The material so washed was completely soluble in hot alcohol, from which it separated on cooling in a bulky, microcrystalline form. On repeating this solution and separation several times the material was obtained nearly free of color. Treatment at this stage with cold petroleum ether removed the remaining color and a small quantity of oily matter. The body after this purification melted at 80–81° C., was soluble in ether and hot alcohol, little soluble in cold alcohol, and insoluble in water. Its elementary composition was found to correspond to the formula $C_{24}H_{48}O_2$.

	Found.	Calculated for $C_{24}H_{48}O_2$.
Carbon	78.1	78.2
Hydrogen	13.2	13.0
Oxygen	8.7	8.8

The compound dissolved in aqueous alkalies and was set free from such solution unchanged on addition of a mineral acid. The properties, melting point, and composition indicate the identity of this compound with lignoceric acid first described as obtained by Hell and Hermanns[b] from the solid residue or "paraffin" of beechwood tar. It is also found[c] as a glyceride in peanut oil.

[a] Zeit. Chem., 1869, p. 65.
[b] Ber. chem. Ges., **13**, 1713 (1880).
[c] Kreiling, Ber. chem. Ges., **21**, 880 (1888).

It is generally assumed that the lignoceric acid found in wood tar is the result of decomposition effected by the method of treatment and that it does not occur as such in the wood. In the case of the soil, however, certain observations indicated that this might not be the case. It was observed that if the soil was heated to a high temperature or the heating done rapidly the yield of solid matter in the distillate was very small. When the heating was done carefully and the temperature raised only to the point where a distillate could be obtained, the solid product seemed to be the result of distillation directly from the soil and when the operation was carried on in a glass tube such distillation could actually be observed, the melted distillate collecting on the upper, cooler side of the tube and being driven forward as heat was applied, just as water would be. It was also found that the purified lignoceric acid obtained from the soil in the manner described could by carefully heating be distilled with little or no decomposition.

With the object of extracting the lignoceric acid from the soil if it existed as such, the soil was treated with boiling 95 per cent alcohol, the extract filtered hot, and allowed to cool. On cooling, a voluminous precipitate separated from the dark-colored extract. This was separated by filtration and purified by dissolving several times in hot alcohol, from which it separated on cooling, and finally by washing with cold petroleum ether. The compound so obtained corresponded in all properties with that obtained by distillation. It melted at 80–81° C., and elementary analysis gave the following figures:

	Found.	Calculated for $C_{24}H_{48}O_2$.
Carbon	78.2	78.2
Hydrogen	13.8	13.0
Oxygen	8.0	8.8

The identity of the two bodies obtained from the soil by distillation and by extraction with hot alcohol is thus established. It follows that lignoceric acid exists in the soil as such.

Regarding the source of the lignoceric acid in the soil there are two possibilities suggested by our knowledge of the compound. It is, as has been already stated, present in peanut oil as a glyceride and may be a component of other vegetable oils and be somewhat widely distributed in plants in small amounts, in which case it might occur in the soil as a residue of the decomposition of such glycerides.

Lignoceric acid is obtained by the distillation of wood, presumably through the decomposition of woody tissue. It is possible that similar decomposition through the agency of micro-organisms may take place in the soil.

RESIN ACIDS.

There have been separated from a peaty soil from North Carolina three fractions having the properties of resin acids. While their identity as single chemical compounds has not been established, there is enough known concerning their properties and composition to warrant their description under this division of acids of unknown constitution.

When the alkaline or humus extract of a soil is acidified, the humus extract filtered off, washed, and treated with boiling 95 per cent alcohol, a portion of the humus precipitate goes into solution. If the alcohol is removed by evaporation and water added to keep the volume constant, a dark-colored precipitate is formed which, after filtration, washing, and drying, is a red or brown resinous powder. This procedure was the preliminary stage in the separation of α-hydroxystearic acid and paraffinic acid, these being removed from this resinous material by boiling petroleum ether.

The resin acids to be described were obtained from a peaty soil, the same from which lignoceric acid was obtained. The treatment was, as just outlined, the extraction of the humus precipitate with hot alcohol, resulting in a resinous mass which was extracted with petroleum ether. The portion insoluble in petroleum ether, which was the greater portion of the precipitate, was then repeatedly treated with boiling ether until no more material was dissolved. The ether solution was then shaken successively with water solutions of alkaline salts in the following order: First, 1 per cent ammonium carbonate solution; second, 1 per cent sodium carbonate solution; and finally, 1 per cent sodium hydroxide solution. The extraction with each solution was repeated till no more material was taken from the ether before the next in order was used. This treatment extracted about 90 per cent of the matter originally soluble in the ether. The several alkaline solutions thus obtained were acidified with hydrochloric acid. The resin acids were set free and being insoluble in water were separated by filtration. They were further purified by dissolving again in ether and repeating the extraction with aqueous solution of the same salts in the same order, the acids being separated, washed, and dried. As so obtained, they were resinous powders varying in color from light yellow to dark orange. These bodies have been designated for purposes of description, resin acid I, II, and III, I having been extracted from ether by ammonium carbonate solution, II by sodium carbonate solution after the extraction of I, and III by sodium hydroxide solution after the extraction of I and II. The melting points of these acids were sharp and were as follows (uncorrected): I, 100° C.; II, 95° C.; III, 190° C. They had the same general appearance and an indefinite crystalline structure under the microscope. They were readily soluble in alcohol and ether and in aqueous solution of sodium or potassium hydrate;

I and II were soluble in ammonia, but III was very slightly so. All gave in alcohol solution a slight reddish color with ferric chloride, but did not respond to Liebermann's cholesterol reaction.

The elementary composition was found to be as follows, the per cent of ash being given, but the composition stated on an ash-free basis:

	Resin acid I.	Resin acid II.	Resin acid III.
Ash	0.40	1.01	0.58
Carbon	68.04	73.73	74.07
Hydrogen	7.74	9.46	9.94
Oxygen	24.22	16.81	15.99

Resin acids II and III gave figures so nearly alike that they might be considered identical were it not for the wide difference in melting point and solubility in ammonia already mentioned. All gave somewhat similar products on fusion with potassium hydrate, a mixture of phenol derivatives, and lower fatty acids.

The method used for the separation of these resin acids is that adopted by Tschirch in the examination of resins. The material left in the ether after shaking with alkalies he designates resin esters. This fraction will be described later. It is evident that in this process there is a separation into fractions that have quite distinct properties and composition in spite of general resemblances. However, the investigation at this stage is quite unsatisfactory in regard to the identity of these several fractions as single organic acids or even as acids at all. It is assumed that because a body goes from solution in ether into solution in an alkaline salt it is a case of a free acid in the ether forming a salt soluble in water. There was, however, in the case of the shaking with carbonates no evolution of carbon dioxide, and the process was not the simple one of a replacement of carbon dioxide and the formation of a salt with the resin acid.

The whole chemistry of the resins and so-called resin acids is in a very unsatisfactory condition. A great many separations have been made similar to those made in this case, named and described as definite compounds, when all that was known was their elementary composition and some of their physical properties.

The class of compounds known as phlobaphanes or anhydrides of tannic acid are closely related to the so-called resin acids in their physical properties, composition, and products formed on fusing with caustic potash. These anhydrides, of which there seems to be a series, adjacent members differing from one another by one molecule of water, are in the same unsatisfactory condition chemically as are the resin acids.

The several members of the phlobaphane series differ in solubility, and any one of the series may change its solubility and other physical properties by loosing water and being transformed into another. There is some evidence that in these fractions designated resin acids there is some such anhydride constitution and ready change of solubility.

There is then in the three fractions here described a separation of a considerable portion of the organic matter of a soil into three separate substances having the same general properties but differing in melting point, composition, and some other properties. No definite conclusion can be stated regarding the identification of these substances, because the chemistry of the natural products to which they are related and with which they must be compared is largely unknown. These fractions form a large proportion of the organic matter and further research into their constitution and nature is necessary.

Nearly all soils examined give by the method described subtsances of similar appearance and properties.

ESTERS AND ALCOHOLS.

This group finds its largest representation in nature in the oils, fats, and waxes of plant and animal life. Practically all the fats and oils are esters of the alcohol glycerol, $C_3H_5(OH)_3$, and may be represented thus: $C_3H_5(OR)_3$, where R is the acid radical of a fatty acid. The acid radical may be of one or more acids, but in most natural fats two acids are represented; for instance, oleo-distearin is $C_3H_5\begin{cases}OC_{18}H_{33}O\\(OC_{18}H_{35}O)_2\end{cases}$. The waxes are usually esters of higher alcohols, such as cetyl, $C_{16}H_{33}OH$, ceryl, $C_{16}H_{51}OH$, cholesterol, $C_{26}H_{43}OH$, with the same higher fatty acids that occur in glycerides. Both oils and fats frequently contain small quantities of the esters classed as waxes. Most of the natural waxes are complex mixtures of esters, higher alcohols, and higher fatty acids, but as in fats and oils the esters are usually the chief component.

From the wide distribution of this class of bodies in plants and animals it follows that considerable quantities of them get into the soil and form the material from which soil organic matter is made. From the evidence at hand in the form of substances isolated from soils, it is clear that in some soils, at least, these compounds persist unchanged. The isolated substances represent esters in the form of glycerides and higher alcohols.

Esters of another class not so well known occur in resins, especially those known as balsam resins. These are generally esters of unknown alcohols with aromatic acids such as benzoic or cinnamic. An

ester of this character was separated in connection with the resin acids described in a previous section.

GLYCERIDES OF FATTY ACIDS.

When the alcoholic filtrate from the lead precipitate of paraffinic acid obtained in the isolation of this body in the manner described in previous pages is freed from lead by hydrogen sulphide and the alcohol evaporated, there is left an orange-colored oil which remains clear and liquid at room temperature, but becomes turbid on cooling, crystalline material finally separating. This oil on examination proved to be a mixture of glycerides of fatty acids, as are most natural oils.

The oil obtained in the manner just described is slightly acid to litmus and has an odor not unlike that of olive oil. Its specific gravity at 26° C. is 0.935. On saponification with alcoholic potash and evaporation of the alcohol a soap was obtained completely soluble in water. When this soap solution was acidified with sulphuric acid the fatty acids were set free and extracted by shaking with ether. The mixture of fatty acids obtained on evaporating the ether was a fatty, semisolid mass from which crystalline leaflets separated on standing. This mass immediately after removal of the ether had an odor suggesting capric acid, but this disappeared in a short time, leaving a tallow-like odor. On shaking the mixed fatty acids with equal parts of nitrosyl sulphuric acid and water, a solid mass was obtained on standing, thus showing the presence of oleic acid. The original glyceride behaved in the same manner, indicating the presence of oleic acid or olein in the oil. The presence of oleic acid or at least some unsaturated acid was confirmed by the fact that the acid mixture when dissolved in carbon tetrachloride decolorized bromine without setting free hydrobromic acid.

In the water solution left after acidifying the soap solution and extracting with ether the presence of glycerin was shown by the usual tests. The acid liquid was evaporated nearly to dryness and mixed with a small quantity of acid potassium sulphate and the mass so obtained heated in a test tube provided with a stopper and delivery tube which dipped beneath the surface of a few cubic centimeters of water in a second tube. The presence of acrolein in the second tube, as a result of the distillation of glycerin, was established by the characteristic odor and by the generation of a pink color when treated with fuchsine aldehyde reagent.[a]

The fact, then, that the oil obtained from the soil is a glyceride is established, first, by the changed character of the material after saponification, and, second, by the presence of glycerin in the saponification products. It is, of course, possible that there is some free

[a] A solution of fuchsine decolorized with sulphur dioxide.

acid present in the oil, but the indications are that such must be small, if any. That the oil is a mixed glyceride is established by the complex character of the fatty acids obtained on saponification, a semisolid mass from which crystalline material separated, oleic acid also being present.

When the soap solution resulting from the saponification of this oil is dried and extracted with petroleum ether and the ether evaporated, there is obtained a small quantity of unsaponified material. This forms a soft, waxlike mass insoluble in water, but soluble in soap solution. This material gave the Liebermann's cholesterol reaction strongly, but it was not possible to obtain from it any definite crystals of any cholesterol body.

The oil obtained from the soil is, therefore, a glyceride of fatty acids containing a small quantity of unsaponifiable material and possibly a small quantity of free acid. As such, then, it corresponds in all particulars with natural glycerides, especially the vegetable oils.

The presence of glycerides similar to natural oils in the soil can perhaps best be explained on the ground that they are unchanged plant residues which have resisted decomposition. Nearly all plants at some stage of their development contain glycerides and they may also be built up by micro-organisms as with other plants. They are probably not formed by purely chemical reactions such as are likely to take place in the soil.

The presence of unchanged plant glycerides in the soil is of interest in view of the number of bodies of a fatty nature which have already been isolated from soils. In discussing the possible origin of dihydroxystearic acid in the soil it was pointed out that oleic acid in the form of a glyceride was a nearly universal plant constituent and that it was possible that dihydroxystearic acid might be formed from it in the soil in somewhat the same way as in the laboratory.[a] Cholesterol-like bodies seem to occur frequently in soils [b] and oleic acid or olein may have some relation to the occurrence of this group, inasmuch as Lifschütz [c] has shown that cholesterol bodies may be formed by the oxidation of oleic acid.

The glyceride just described was obtained from the Elkton silt loam as the final step in the treatment which resulted in the isolation of α-hydroxystearic acid and paraffinic acid. So far all soils examined in this manner give at this stage an oily, semisolid mass similar in appearance to that obtained from the Elkton silt loam. In only a few cases, however, has sufficient material been obtained to establish its identity as a glyceride.

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[a] Bul. 53, Bureau of Soils, U. S. Dept. Agr. (1909); Jour. Am. Chem. Soc., **30**, 1599 (1908).

[b] Jour. Am. Chem. Soc., **31**, 116 (1909).

[c] Zeit. physiol. Chem., **55**, 1 (1908).

PHYTOSTEROL, $C_{26}H_{44}O.H_2O$.

When the alcoholic extract of the peaty soil, from which lignoceric acid separated on cooling in the manner described in discussing that compound, is carefully evaporated and kept at constant volume by the addition of water until the alcohol is removed there is formed a reddish-brown precipitate insoluble in water. This, after separation and drying, forms a red or brown powder of a resinous nature. On extracting this resinous material with boiling petroleum ether there is obtained on removal of the ether a light-colored, oily residue which becomes semisolid on standing. This oily mass was saponified with alcoholic potash, the alcohol removed, and the soap dried and extracted with boiling petroleum ether. The petroleum ether was removed from this extract and then treated with a relatively large volume of hot alcohol in which it was completely soluble. On cooling there was a separation of some microcrystalline material,[a] which was removed by filtration and the alcohol allowed to evaporate. The residue thus obtained was dissolved in a small quantity of chloroform and allowed to stand. After a few hours crystals having the characteristic appearance of phytosterol, the "cholesterol body" of plants, were formed. The form of these crystals, shown in the photomicrograph, is characteristic of this body and is quite distinct from cholesterol itself or from agrosterol, another cholesterol body found in soils.[b]

The compound obtained in this way, when purified by several recrystallizations melted at 135° C., the melting point of phytosterol being given by different authorities from 132° C. to 138° C. It gave the Liebermann cholesterol reaction strongly, also the cholesterol reaction with sulphuric acid and chloroform. The method by which the body was obtained, its crystalline form, its melting point, and color reactions are sufficient to establish its identity as phytosterol. It should be noted in connection with the isolation of this body that it was obtained as the result of saponification and it has not been possible to obtain it from the peat soil by any method which did not involve saponification, showing that the phytosterol is present in combination, probably as an ester of a higher fatty acid. Phytosterol is found in nearly all vegetable oils, except olive and palm oils, and is obtained from such sources by saponification and is, therefore, probably present in such oils likewise as an ester. The well-known wide distribution of phytosterol in plants and the fact that when found in the soil it is evidently in combination as in plant fats and oils makes it more than probable that this compound is a plant residue remaining undecomposed in the soil.

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[a] Identified as hentriacontane.

[b] Bul. 53, Bureau of Soils, U. S. Dept. Agr. (1909); Jour. Am. Chem. Soc., 31, 116 (1909).

In nearly all soils examined there are obtained at some stage of the investigation unsaponified residues which give Liebermann's cholesterol reaction, but, except in the case of agrosterol previously described and the phytosterol under discussion, it has not been possible to isolate any definite crystalline cholesterol body. There are a number of bodies of a resinous or unknown nature which give some of the cholesterol reactions. These are particularly abundant in the waxy coating of fruits and some of them have been described and given names. Vitin [a] $C_{20}H_{32}O_2$ is one of these compounds, but nothing is known of their constitution.

Agrosterol, another cholesterol body isolated from soils, has been described in the previous bulletin and was obtained by a different method than that used for phytosterol, one which did not involve saponification. It is quite evident from the manner in which this compound was isolated that it must occur as such in the soil. The fact that agrosterol differs in melting point and crystalline appearance from any cholesterol body known to exist in natural products, and the fact that it is free and not combined as in natural products, gives some weight to the theory that it may be formed from some other compound such as a fatty acid through the agency of microorganisms or chemical oxidation.

RESIN ESTERS.

In the separation of the so-called resin acids described in a previous section, after shaking the ether solution with the several solutions of alkaline salts, there was left a small quantity of material in the ether. This fraction according to Tschirch contains resin esters.

The material left after evaporation of the ether was a resinous brown powder melting at 95° C. insoluble in water and aqueous alkalies, but readily soluble in alcohol and ether. It contained 0.98 per cent ash, and its elementary composition stated on the ash free basis was found to be:

	Per cent.
Carbon	68.88
Hydrogen	10.24
Oxygen	20.88

On saponification with alcoholic potash the resulting soap was not completely soluble in water. The saponification mixture was suspended in hot water, acidified with sulphuric acid, and filtered from the insoluble products formed. The filtrate after cooling was

[a] Monatsh. f. Chem., 14, 719 (1893).

shaken with ether and the ether evaporated. The residue so obtained was in part crystalline, the crystals having the appearance and properties of cinnamic acid. The identity of this crystalline body has, however, not been satisfactorily established and is a small part only of the saponification products.

The other products are for the most part amorphous and a treatment of the dried mass with petroleum ether separated it into two portions of different appearance and properties, showing that the original material had suffered a change by saponification and can, therefore, be considered as an ester.

CARBOHYDRATES.

Carbohydrates, represented by sugars, starch, and compound celluloses, make up by far the greater portion of the organic substance of plants and so form the greater part of the material from which soil organic matter is made. The extreme susceptibility of these bodies to fermentation or decomposition by micro-organisms render them probably the most unstable organic material added to soils. Sugars and starch are used by a great variety of bacteria and fungi as food and no doubt disappear as such long before the cell tissues break up and become part of the soil. The cell wall and fibro-vascular tissues are made up for the most part of a complex compound containing a carbohydrate or cellulose radical. These resist the action of micro-organisms longer and in the breaking up of the tissues as a result of the decay of the least resistant portions, this complex probably gets into the soil to some extent unchanged.

The products of decomposition of such carbohydrates as starch and sugars are fairly well known and are for the most part simple compounds, such as alcohol, acetic acid, and carbon dioxide. The more resistant complex, the so-called compound celluloses, contain other groups in addition to the carbohydrate radical and can give rise in decay to a great variety of products some of them still quite complex, but resistant to further action of bacteria or fungi. Because of this complexity of the material itself and of its decomposition products, the character of the changes which take place in the decay of this material, either in or out of the soil, are very imperfectly known.

The isolation of carbohydrates from soil is at present confined to a single compound, a pentosan or a body yielding a pentose sugar.

PENTOSAN, $C_5H_8O_4$.

Nearly all soils when boiled with hydrochloric acid of moderate strength give furfurol. The evolution of furfurol when an organic mixture is heated with hydrochloric acid is usually regarded as

evidence of the presence of pentosans. Pentosans are bodies of a gummy nature and unknown constitution for which the formula $C_5H_8O_4$ is usually given, that give on heating with acids pentose sugars, $C_5H_{10}O_5$, which are definite crystalline bodies of known constitution. These pentose sugars on further heating with acids give furfurol, the amount of furfurol being proportional to the amount of pentosan or pentose. Based on this fact a method has been devised for determining the pentosan by determining the amount of furfurol obtained under certain conditions. This method is adopted as a provisional one by the Association of Official Agricultural Chemists.[a]

The method, when applied to soils, generally gives weighable quantities of the phloroglucid, the form in which the furfurol is determined. The figures given below show results obtained by this method applied to ten soils of widely different types and character of organic matter. Ten grams of soil were used, boiled with 12 per cent hydrochloric acid and the distillation carried on in the usual manner until there was no further evolution of furfurol, which was usually the case when the distillate amounted to 300 or 400 c. c. The furfurol was determined as phloroglucid and the calculation made according to the formula adopted in the provisional method cited.

Pentosan in soils and relation to total carbon.

Soil.	Per cent total carbon.	Per cent pentosan.	Per cent pentosan carbon.	Pentosan carbon per 100 of total carbon.
Elkton silt loam	0.522	0.176	0.079	15.13
Sassafras silt loam	.315	.055	.025	7.93
Chester silt loam	1.510	.182	.083	5.49
North Carolina peaty soil	27.102	1.090	.495	1.83
Marshall loam	6.971	2.750	1.249	17.93
California peaty soil	11.478	.341	.150	1.30
Norfolk fine sandy loam	.822	.027	.012	1.46
Santa Paula, Cal., loam	1.308	.061	.028	2.14
Portsmouth loam	3.854	.219	.099	2.57
Susquehanna clay loam	1.048	.659	.299	28.53

The figures given here illustrate well, perhaps better than any others available, the great variation of organic matter in soils. In these 10 soils selected somewhat at random the pentosan carbon per 100 of total carbon varies from 1.30 to 28.53, while the third lowest result, 1.83, was obtained from a soil containing the largest quantity of organic matter.

The Marshall loam, containing the largest quantity of pentosan, 2.75 per cent, was selected for further investigation. An alkaline extract of this soil was made by treating it for several hours with 2

[a] Bul. 107 (Revised) Bureau of Chemistry, U. S. Dept. Agr., p. 54.

per cent sodium hydrate, the dark-colored extract siphoned off, and the humus bodies precipitated by acidifying with acetic acid, the filtrate made neutral and filtered from the precipitate formed. Lead acetate in excess was added to the neutral filtrate, resulting in the formation of a heavy brown precipitate and the removal of nearly all the color from the solution. To the filtrate from this precipitate dilute ammonia was carefully added, when a light yellow, voluminous precipitate was formed. This, after washing, was suspended in water and treated with dilute sulphuric acid, avoiding excess. The lead sulphate was removed by filtration and the filtrate concentrated to a small volume. To this solution three volumes of 95 per cent alcohol were added, when a gummy precipitate was formed which, after washing with alcohol, dried to a hard, translucent mass. This mass contained traces of lead which it retained very persistently. It was soluble in alkalies and formed an opalescent mucilage with water. On heating with 12 per cent hydrochloric acid, furfurol was given off freely and on heating with phloroglucin and hydrochloric acid the characteristic purple color reaction for pentose sugars was obtained. On digesting for some time with sulphuric acid of moderate strength and neutralizing, a solution was obtained which reduced Fehling's solution and gave an osazone melting at 160° to 161° C. On treating with cadmium carbonate and bromine according to the method of Bertrand,[a] the characteristic crystals of the double salt cadmium xylonate and cadmium bromide were obtained, showing the presence of xylose as one of the products of hydrolysis of the substance isolated from the soil. Tests made for the presence of other pentose sugars gave negative results.

The quantitative method of determining pentosans depends, as has been noted, on the formation of a pentose from the pentosan and the formation of furfurol from the sugar on further heating with acid. In the cell wall material, which makes up such a large portion of the organic matter of plants, there is no doubt a pentosan body present, either as such or as part of the complicated molecule of lignocellulose, for a crude pentosan can be prepared from such material. In the case of the Marshall loam such a crude pentosan was obtained and was probably present as such in the soil as a plant residue. In the case of soil organic matter in general, however, it can not be assumed that the formation of a pentose sugar, and from it furfurol, necessarily indicates the presence of a pentosan as such. Pentose sugars are part of the complicated molecules of nucleo-proteids and phosphatids and are split off from these on heating with acids. The nucleo-proteids are characteristic of tissues in which nucleated cells are abundant and those of animal origin have been especially studied. The investigation of those of plant origin is not so thorough, but

[a] Bul. Soc. Chim. (3), 5, 556 (1891).

there is every evidence that the parts of plants rich in cells are also rich in nucleo-proteids and this compound and its decomposition products must contribute to the organic matter of the soil.

With this in mind it is evident that a determination of pentosan in soil by the method in general use is simply the determination of the furfurol that may arise from a pentosan, a pentose, or a pentose yielding material other than a pentosan.

In the light of our present knowledge of pentosans and pentose-yielding material, their presence in a soil must be regarded either as a plant residue which has resisted decomposition, such as a portion of the lignocellulose, or as products of decomposition of complicated bodies, such as nucleo-proteids.

NITROGENOUS COMPOUNDS.

In the previous sections organic compounds containing carbon, hydrogen, and oxygen only have been discussed. All living matter has as essential components some organic compounds that contain nitrogen in addition to carbon, hydrogen, and oxygen. In consequence, soils also contain compounds of the same character or those arising from them through decomposition. In organic life the nitrogenous compounds are for the most part those related to protein, either those from which protein is built up or those resulting from its decomposition. In these some of the nitrogen is always linked with hydrogen in the amino form, NH_2, some of it in more complex compounds being in the imino, NH, form. In consequence of this structure, protein itself and its nitrogenous decomposition products are either basic or are what may be called potential bases.

In the decomposition of protein there are two groups of nitrogenous compounds, the monamino and the diamino acids, also called hexone bases. Both contain the carboxyl group COOH and in the monamino acids there is but one NH_2 group and the compounds are mainly acid in character. Many of them, however, have the property of forming compounds with both acids and bases. Glycocoll, CH_2NH_2COOH, leucine, $CH_2(CH_2)_3CHNH_2COOH$, and glutaminic acid, $COOH(CH_2)_2CHNH_2COOH$ are examples of this monamino acid group.

In the diamino acids or hexone bases there are two or more NH_2 groups and sometimes an NH group and the basic character usually predominates; lysine, $CH_2NH_2(CH_2)_3CHNH_2COOH$ and diamino propionic acid, $CH_2NH_2CHNH_2COOH$, are examples of this group.

In addition to protein, which breaks up into amino acids, there are in the cell nucleus what are known as nucleo-proteids, already referred to in discussing pentose yielding material. These nucleo-proteids are combinations of protein with a nonprotein radical. This nonprotein radical, known as nucleic acid, is a complex body of

unknown constitution, but its decomposition products are well known. These are: Phosphoric acid, a characteristic and constant product; pentose sugar, usually present; lævulinic acid, sometimes present; pyrimidine derivatives, always present; and purine bases, always present. These last two groups are nitrogenous compounds and differ in several respects from the amino acids. They contain no carboxyl, they are of ring formation, and the nitrogen may be either uncombined, or combined with hydrogen, as NH or as NH_2.

The pyrimidine derivatives known as decomposition products of nucleic acid are uracil, $C_4H_4N_2O_2$, cytosine, $C_4H_5N_3O$, and thymine, $C_5H_6N_2O_2$. The other group, the purine bases, are derivatives of a body purine which in its turn is closely related to pyrimidine. The chief members of this group are xanthine, $C_5H_4N_4O_2$, hypoxanthine, $C_5H_4N_4O$, adenine, $C_5H_5N_5$, and guanine, $C_5H_5N_5O$.

Among the nitrogenous compounds isolated from soils there are representatives of three of the above-mentioned groups resulting from the decomposition of protein or nucleo-proteids, viz, diamino acids, pyrimidine derivatives, and purine bases.

To the nitrogenous groups, here mentioned as having been found in soils, must be added the pyridine group represented by the picoline carboxylic acid, the presence of which in soils was reported upon in the earlier work cited.

DIAMINO ACIDS (HEXONE BASES).

All protein bodies give some diamino acids on decomposition. Three of these occur very frequently, and usually together, but in varying proportions, one predominating in one protein and another predominating in a protein from some other source. These are lysine, arginine, and histidine, all basic compounds the constitution of which is known. Two of these, arginine and histidine, have been isolated from soils.

HISTIDINE, $C_6H_9O_2N_3$.

Histidine and arginine usually occur together, and the method of their isolation depends on the fact that they are precipitated by silver salts, sulphate or nitrate, from alkaline solution. The solution may be made alkaline with ammonia or barium, but the fact that the histidine silver precipitate is dissolved by a slight excess of ammonia makes the use of barium hydroxide preferable. Separation of the two is accomplished by taking advantage of the fact that histidine silver is precipitated in slightly alkaline solution, while the arginine compound is not precipitated except in strongly alkaline medium. The isolation and separation of these two compounds is then simply two steps in one method. The method in detail is as follows:

FIG. 1. PHYTOSTEROL.

FIG. 2. XYLOSE OSAZONE.

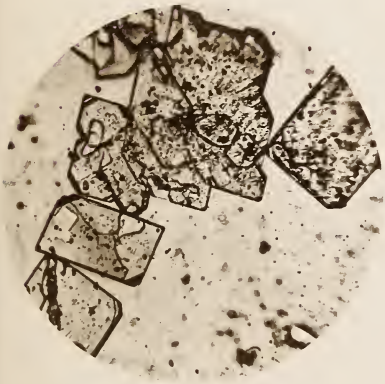


FIG. 3 HISTIDINE DIHYDROCHLORIDE

FIG. 4. HYPOXANTHINE HYDROCHLORIDE

FIG. 5. CYTOSINE

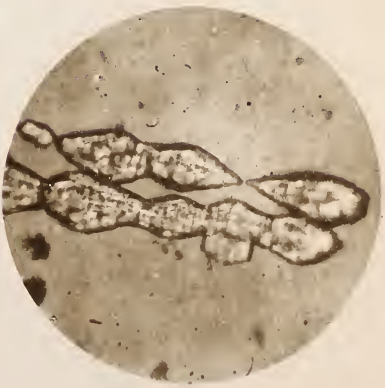


FIG. 6 CYTOSINE CHLOROPLATINATE.

PHOTOMICROGRAPH OF ORGANIC COMPOUNDS ISOLATED FROM SOILS AND OF CHARACTERISTIC SALTS OR DERIVATIVES OF THESE

The soil, Houston clay, was treated with 2 per cent sodium hydroxide solution for several hours, the clear liquid siphoned off and acidified with sulphuric acid. In the case of this soil, which was calcareous, the alkaline extract was light colored and, on acidifying, the humus precipitate was slight. The acid filtrate was made neutral and to it without filtering silver sulphate in excess was added and then filtered. From this point the method of Kossel and Kutscher [a] was used. The neutral filtrate was saturated with barium hydroxide and the precipitate removed and washed with barium hydroxide solution. The precipitate of arginine and histidine silver was then suspended in dilute sulphuric acid and decomposed with hydrogen sulphide. The filtrate from the silver sulphide was then evaporated to a smaller volume, neutralized with barium hydroxide, and barium nitrate added until all sulphuric acid had been precipitated. The filtrate from barium sulphate was then concentrated and silver nitrate added until a drop added to an excess of barium hydroxide solution gave a yellow precipitate. The solution was then neutralized with barium hydroxide and the addition carefully continued until a portion of the liquid no longer gave a precipitate with ammoniacal silver nitrate, showing complete precipitation of histidine. The arginine remains in solution.

The histidine silver precipitate, after being filtered off, washed, and suspended in dilute sulphuric acid, was decomposed with hydrogen sulphide. The filtrate from the silver sulphide was freed from sulphuric acid with barium hydroxide and excess of the latter removed with carbon dioxide. The filtrate from barium carbonate was evaporated to dryness, taken up with silver nitrate solution and a drop of nitric acid and filtered from any insoluble matter present. Ammoniacal silver nitrate was then added, the precipitate filtered off, washed, and decomposed on the filter paper with dilute hydrochloric acid. The filtrate from the silver chloride contains the histidine as hydrochloride, and on concentration to a small volume the histidine crystallizes out as the dihydrochloride in characteristic glassy plates or prisms. The crystals formed are well developed, even when but a small quantity of histidine is present, and can easily be separated and purified by recrystallizing from dilute hydrochloric acid. The crystals are quite characteristic and a crystallographic description has been given by Schwantke,[b] and Kossel.[c]

The characteristic form of the crystals and the method by which the compound was obtained are sufficient to establish its identity as

[a] Zeit. physiol. Chem., **31**, 166 (1900).
[b] Zeit. physiol. Chem., **29**, 491 (1900).
[c] Zeit. physiol. Chem., **22**, 182 (1896).

histidine dihydrochloride and can be confirmed by two color reactions. Knoop's color reaction: To a solution of the histidine salt add bromine water until the yellow color produced is permanent. On heating the color disappears, but is replaced shortly by a faint red, which gradually passes to a deep red, amorphous particles separating, and the liquid becoming turbid. This reaction is not very delicate and is interfered with by the presence of free alkali or excess of bromine. Pauly's diazo reaction:[a] Histidine or its salts in alkaline solution give with diazo-sulphanilic acid a red color which does not disappear on dilution. Tyrosine also gives a similar color, but histidine or its salts can not be confused with tyrosine in crystalline form, the latter crystallizing in needles that are nearly insoluble in water. This reaction is much more delicate than the one with bromine—1 part in 100,000 is said to give a distinct pink color.

The histidine dihydrochloride obtained from the soil gave both these color reactions, the latter very definitely.

The histidine dihydrochloride obtained by this method is easily soluble in water, crystallizes without water of crystallization, and melts at 231° C. with some charring below this point. The free base can be obtained from the dihydrochloride by decomposition with silver sulphate. It is not very soluble in water and crystallizes in plates or needles. It is faintly alkaline in reaction, but does not take up carbon dioxide.

ARGININE, $C_6H_{14}O_2N_4$.

The method of isolating arginine, which was simply a further step in the method used in the isolation of histidine, was as follows: The filtrate from the histidine silver precipitate was saturated with powdered barium hydroxide, the precipitate filtered off, washed with barium hydroxide solution, suspended in dilute sulphuric acid, and decomposed with hydrogen sulphide. The filtrate from the silver sulphide was freed from sulphuric acid with barium hydroxide and the filtrate from excess of barium with carbon dioxide. The filtrate from the barium carbonate was neutralized with nitric acid and evaporated to a small volume when arginine nitrate crystallized out. The neutral nitrate, $C_6H_{14}O_2N_4HNO_3.H_2O$, thus obtained crystallizes from water in opaque masses composed of minute needles. When anhydrous it melts at about 175° C., but not sharply. It is easily soluble in water, easily in hot alcohol, but with difficulty in cold. An acid nitrate, $C_6H_{14}O_2N_42HNO_3$, can be obtained by evaporating the neutral nitrate with excess of nitric acid. It crystallizes without water of crystallization in long needles or plates and melts at 145° C. The free base can be obtained by adding silver nitrate solution to the acid nitrate solution and then treating this with sodium hydroxide

[a] Zeit. physiol. Chem., 42, 508 (1904).

solution, when arginine silver is precipitated. This is decomposed with hydrogen sulphide. The filtrate from the silver sulphide on concentration deposits the free base in rosettelike masses of plates melting at 207° C.

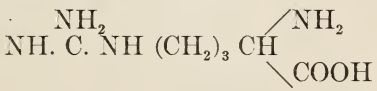
The arginine obtained from the soil as neutral nitrate by the method described[a] was found to conform to the description of this salt, as did also the acid nitrate and free base made from it.

The method by which it was obtained and this conformity in crystalline form, melting point, and other properties are sufficient to establish the identity of the compound obtained as arginine.

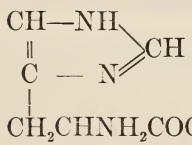
The quantity of arginine obtained from the Houston clay soil was much smaller than the quantity of histidine, and histidine has been obtained from several other soils from which no arginine could be obtained.

The isolation of histidine and arginine from soils has several points of theoretical interest. They are basic compounds capable of neutralizing acids. The compounds resulting from the decomposition of organic matter are commonly thought of as essentially acid in character, and it is true that practically all that arise from carbohydrates are acid in character, as are also the majority arising from the decomposition of fats. However, the great majority of those derived from the decomposition of protein, while not actually basic as are arginine and histidine, are, when nitrogenous, potentially basic in that they contain the amino, NH_2, group. Even when the acid character predominates, as in monamino acids, they can unite with strong acids to form salts.

The constitution of both arginine and histidine is known. Arginine is guanidine α-amino normal valerianic acid.[b]



Histidine has the following constitution:[c]



[a] Gulewitsch, Zeit. physiol. Chem., **27**, 189 (1899).

[b] Schulze and Winterstein, Zeit. physiol. Chem., **26**, 1 (1898); Ellinger, Zeit. physiol. Chem., **29**, 334 (1900); Kutscher, Zeit. physiol. Chem., **32**, 278 (1901); **32**, 413 (1901); **42**, 93 (1904); Fischer, Ber. chem. Ges., **34**, 454 (1901).

[c] Pauly, Zeit. physiol. Chem., **42**, 513 (1904); Knoop and Windaus, Beitr. chem. Physiol. Path., **7**, 144 (1906); **8**, 406 (1906); Knoop, Beitr. chem. Physiol. Path., **10**, 111 (1907).

Histidine is then an imidazole derivative and contains but one amido NH_2 group, and is not a diamino acid. It has, however, been so long classed with the diamino acids that it is included in that group here.

Both have, as have all amino acids derived from protein, an amino group in the α position.

PYRIMIDINE DERIVATIVES.

Some of the pyrimidine bases, uracil, cytosine, and thymine are constant products of the decomposition of the nucleic acid of nucleoproteids, and while all three may be present as the result of the decomposition of nucleic acids of animal origin, thymine seems to be absent from those of plant origin.

The plant nucleic acids studied are as yet few in number. That of yeast is the best known and has been extensively investigated by Kossel.[a] Osborne and Harris[b] have isolated a nucleic acid from the wheat embryo which seems to be identical with yeast nucleic acid. Klinkenberg[c] obtained from palm cake and poppy seed nucleic acid resembling that of yeast in composition, and there is every reason to conclude that in plants, as in animals, all parts rich in cells are rich in nucleic acids.

In addition to the nucleic acids of higher plants, which may make up a portion of the organic matter of soils, the micro-organisms which are so abundant in soils contain and probably elaborate nucleoproteids from other nitrogenous material. Galeotti[d] found nucleoproteid in bacteria and Stutzer[e] has shown that 40 per cent of the nitrogen of molds is nuclein nitrogen.

There are, then, two ways in which nucleic acids and their decomposition products may become part of the soil: From the introduction of the nucleic acids of higher plants on the death and decay of the same in the soil; and from nucleo-proteids elaborated in the soil from other nitrogenous compounds by micro-organisms.

CYTOSINE, $C_4H_5ON_3.H_2O$.

A crystalline compound identified as the pyrimidine base, cytosine, has been isolated from several soils by the following method: The soil was treated for several hours with 2 per cent sodium-hydroxide solution, allowed to stand, and the dark-colored extract siphoned off. This was acidified with dilute nitric acid and filtered from the humus precipitate. The acid filtrate was then neutralized with sodium

[a] Zeit. physiol. Chem., **3**, 284 (1879); **4**, 290 (1880); **7**, 7 (1882).
[b] Zeit. physiol. Chem., **36**, 85 (1902); Jour. Am. Chem. Soc., **22**, 379 (1900).
[c] Zeit. physiol. Chem., **6**, 155 (1882).
[d] Zeit. physiol. Chem., **25**, 48 (1898).
[e] Zeit. physiol. Chem., **6**, 572 (1882).

hydroxide and filtered from the precipitate formed. The neutral filtrate was then treated with an excess of mercuric nitrate and the whole brought to neutrality again with sodium hydroxide. The precipitate formed was filtered off, washed, and decomposed by hydrogen sulphide. The filtrate from the mercuric sulphide was concentrated to a small volume and silver nitrate added in slight excess and filtered from the colored precipitate formed. To the filtrate dilute ammonia was carefully added until a precipitate was no longer formed. An excess of ammonia was avoided since the precipitate is dissolved thereby. The silver precipitate was separated by filtration, washed, and suspended in hot water, and decomposed by hydrogen sulphide. The filtrate from the silver sulphide was concentrated to a small volume and allowed to stand, when crystals formed in a short time.

These crystals were in the form of needles or thin shining plates with uneven faces. The compound was purified by recrystallizing several times from water. Thus prepared, the compound is in the form of clear crystals, which on exposure to the air lose water and become opaque. It does not melt at 300° C., but darkens slightly. It is difficultly soluble in cold water, but readily in hot, from which solution it rapidly separates on cooling. The relative solubility in hot and cold water is influenced by impurities which generally accompany the first crude separation. It is difficultly soluble in alcohol and insoluble in ether. The water solution is neutral in reaction, but compounds of definite crystalline appearance are formed with mineral acids. The sulphate crystallizes in needles and the hydrochloride in prisms. Addition of sodium picrate to a water solution forms a difficultly soluble picrate, crystallizing in needles. A difficultly soluble chloroplatinate is formed, crystallizing in characteristic prisms. Addition of potassium bismuth iodide to an acidified water solution forms a red micro-crystalline precipitate.

These reactions, the crystalline appearance of the compound, and the method by which it was obtained establish its identity as cytosine. The identification was further confirmed by the following determinations: A pure preparation crystallized from water, dried a short time between filter paper, lost water of crystallization at 100° C.

	Found.	Calculated for $C_4H_5N_3O + H_2O$.
Water of crystallization	13.79	13.97

It was found to contain nitrogen corresponding to the formula for cytosine:

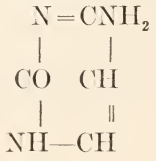
	Found.	Calculated for $C_4H_5N_3O$.
Nitrogen	37.92	37.84

These figures are sufficient to make the identification certain.

A modification of the method by which cytosine was obtained that has some advantages is as follows: Acidify the alkaline-soil extract with sulphuric acid, filter from the humus precipitate, and to the acid filtrate add a solution of mercuric sulphate in 5 per cent sulphuric acid, using an excess. Filter from any precipitate that may be formed immediately and allow the filtrate to stand several days. The precipitate formed should contain the cytosine. This precipitate, after being filtered off and washed, is decomposed with hydrogen sulphide and the filtrate treated with silver nitrate as in the method first described.

The cytosine can also be obtained from the neutral solution obtained as in the first method without the preliminary precipitation with mercuric nitrate by treating the neutral solution with silver nitrate in the manner described. In this case, however, the final solution contains histidine, if any is present in the soil. Histidine does not interfere with the crystallization of the cytosine, but other unknown impurities, which the mercuric-nitrate precipitation removes, accompany the two.

Cytosine was discovered by Kossel and Neumann [a] among the products resulting from treating yeast nucleic acid with sulphuric acid. Its constitution was determined by Kossel and Steudel [b] and it was made synthetically by Wheeler, Johnson, and Jamieson.[c] Its structure is represented by the formula



The closely related compounds uracil, $C_4H_4O_2N_2$, and thymine, $C_5H_6O_2N_2$, as has been stated, are also obtained by the decomposition of nucleic acids. Cytosine while obtained from nucleic acids of

[a] Ber. chem. Ges., **27**, 2215 (1894).

[b] Zeit. physiol. Chem., **37**, 177, 377 (1902); **38**, 49 (1903).

[c] Am. Chem. Jour., **29**, 492 (1903); **32**, 342 (1904).

various origin seems to take the place of thymine in those of vegetable origin so far as they have been investigated. In addition to being obtained from yeast-nucleic acid, it has been obtained from the nucleic acid of the wheat embryo.[a]

The nucleic acids are not split into the pyrimidine derivatives and other decomposition products by the proteolytic enzymes, such as pepsin and trypsin, but this cleavage can be brought about by erepsin and other closely related enzymes which have been called nucleases.[b] Micro-organisms also effect a similar decomposition.[c] From this it would seem that the decomposition products of nucleic acids should be of as constant occurrence in soils as nucleo-proteids are in plants.

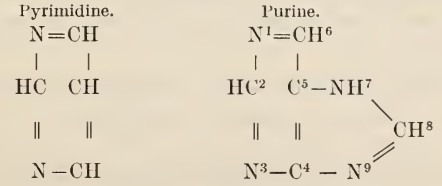
The soil from which cytosine was isolated in the manner described was the Marshall loam, a soil that has been described in connection with the isolation of other compounds. Crystals similar in appearance and properties have been isolated by the same method for several other soils and the indications are that pyrimidine derivatives are present as part of the organic matter of many soils.

PURINE BASES.

The purine bases, xanthine, hypoxanthine, adenine, and guanine, occur as such both in plant and animal tissues, but so far as known are always derived from the breaking down of nucleo-proteids. Three other purine bases, caffeine, theobromine, and theophylline, are the physiologically active principles in tea, coffee, and a number of other plants used as stimulants.

The purine bases are all derivatives of a body, purine, $C_5H_4N_4$, which has not been found in nature. The constitution of these bases is known and they have been made synthetically.[d]

As has been noted, purine is closely related to pyrimidine, and this relationship may be expressed by saying that purine contains a pyrimidine remainder. This relation is shown by the structural formulas:



[a] Wheeler and Johnson, Am. Chem. Jour., **29**, 505 (1903).

[b] Nakayama, Zeit. physiol. Chem., **41**, 348 (1904); Iwanoff, Zeit. physiol. Chem., **39**, 31 (1903); Sachs, "Ist die Nuklease mit dem Trypsin identisch." Inaug. Dissert. Heidelberg. 1905.

[c] Schittenhelm and Schröter, Zeit. physiol. Chem., **41**, 284 (1904).

[d] Fischer, Ber. chem. Ges., **32**, 435 (1899).

The constitution of the four purine bases mentioned and their relation to purine and each other is expressed as follows:

Xanthine is 2. 6. oxypurine;
Hypoxanthine is 6. oxypurine;
Adenine is 6. aminopurine;
Guanine is 2. amino 6. oxypurine;

and they are also related to uric acid, which is 2.6.8. trioxypurine.

The purine bases are susceptible to the action of certain enzymes and of micro-organisms. It was shown by Schittenhelm and Schröter [a] that putrefactive bacteria, especially the coli-bacillus, were able to convert one purine base into another. In the animal organism it has been shown by Burian [b] that xanthine and hypoxanthine are oxidized to uric acid by an oxidizing enzyme. Guanine and adenine can not, however, be oxidized directly in this way, but must first be deaminized by enzymes of another character, being converted thereby to xanthine and hypoxanthine, respectively.[c]

The same transformation of guanine and adenine into xanthine and hypoxanthine has been observed by Schindler [d] in putrefaction.

On the other hand, Kutscher [e] found that in the sterile autodigestion of yeast xanthine and hypoxanthine soon disappeared, leaving only guanine and adenine.

It would seem, then, that the purine bases are very susceptible to change one to another through the activity of enzymes or micro-organisms. Since investigation has shown some of these bases in the majority of soils examined for them, it may be that further investigation will establish some relation between the presence of some one of these bases and the presence of some particular micro-organism or combination of biological factors.

Two members of this group, xanthine and hypoxanthine, have been isolated from soils.

XANTHINE, $C_5H_4O_2N_4$.

From the Marshall loam, containing 6.9 per cent organic carbon and 0.55 per cent total nitrogen, xanthine has been obtained by the following method:

An extract of the soil was made in the manner used in the isolation of a number of other compounds, treating with 2 per cent sodium hydroxide. The dark-colored extract, after allowing the soil to settle, was siphoned off and the alkaline liquid treated according to the

[a] Zeit. physiol. Chem., **39**, 203 (1903).
[b] Zeit. physiol. Chem., **43**, 497 (1905).
[c] Jones and Austrian, Zeit. physiol. Chem., **48**, 110 (1906); Jour. Biol. Chem., **3**, 227 (1907).
[d] Zeit. physiol. Chem., **13**, 432 (1889).
[e] Zeit. physiol. Chem., **32**, 66 (1901).

method of Balke [a] for the estimation of purine bases. Fehling's solution, 10 c. c. to each liter, was added and the solution heated to boiling. A few drops of a dilute solution of hydroxylamine hydrochloride were then added, and this continued until the precipitate formed was red in color. The precipitate was separated by filtration, washed, and suspended in a fairly large volume of water, brought to boiling, and treated while hot with hydrogen sulphide. The filtrate from the copper sulphide was then concentrated on the water bath. As this solution became concentrated a film or pellicle of white microcrystalline material formed on the surface, and when the liquid was reduced to a small volume this was removed by filtration. The substance so obtained, after purification by re-solution in hot water and separation on cooling, was a white microcrystalline powder, little soluble in cold water, but easily soluble in aqueous alkalies. A small quantity evaporated with nitric acid left a yellow residue that on addition of sodium hydroxide and heating became yellowish-red and finally purple, the well-known xanthine reaction.

On dissolving the substance in ammonia and adding ammoniacal silver nitrate, a gelatinous precipitate was formed. This was soluble in hot dilute nitric acid (sp. gr. 1.1), and on cooling this solution there was no separation except on very long standing. This behavior with ammoniacal silver nitrate distinguishes xanthine from other members of the group and is used as a means of separation when they occur together. The ammoniacal silver nitrate precipitate is treated with boiling nitric acid (sp. gr. 1.1) when the xanthine and hypoxanthine precipitates dissolve, leaving adenine and guanine. From the solution so obtained hypoxanthine silver nitrate separates immediately on cooling. The xanthine remains in solution, and separates only, if at all, after long standing.

The substance obtained from the soil formed crystallized compounds with hydrochloric and nitric acids, the latter having the characteristic crystalline appearance of xanthine nitrate. On adding acetic acid to a very dilute solution of the compound in ammonia and allowing to stand several days, groups of thin rhombic plates were obtained having the characteristic appearance of xanthine obtained under these conditions. The method of isolation and the reactions described fix the compound as a purine base and identify it as xanthine.

Instead of treating the alkaline soil extract as described, xanthine or other purine bases can be separated with advantage, in some cases by treatment of the acid solution resulting from acidifying the alkaline extract and filtering off the humus precipitate. This solution, made acid preferably with sulphuric acid, is heated to boiling,

[a] Jour. prakt. Chem. [2], **47**, 537 (1893).

solution of copper sulphate added, and then a small quantity of a saturated solution of sodium bisulphite. The precipitate formed is filtered off and treated in the same manner as was the precipitate obtained with Fehling's solution. The advantage of the use of copper sulphate and sodium bisulphite lies in the fact that they can be used in neutral, acid, or alkaline solution.

Xanthine was first found in a urinary calculus in 1817, and since then has been found to be widely distributed in the tissues of both plants and animals. It has been found present as such in yellow lupines, sprouts of malt, in sprouts of lupines, in vicia sativa, in cucurbita, in phaseolus, and in tea leaves, and no doubt further investigation will disclose its presence in many other plants.[a]

Xanthine then may get into the soil by the death and decay of plants that contain it as such, but it is probable that the most constant source of this, as well as other purine bases, is in the decomposition of nucleic acid and of nucleo-proteids in the soil.

HYPOXANTHINE, $C_5H_4ON_4$.

Hypoxanthine, another purine base, has been isolated from a number of soils by one of the methods outlined in discussing xanthine. The alkaline soil extract made with 2 per cent sodium hydroxide was made slightly acid with sulphuric acid and filtered from the humus precipitate. Copper sulphate solution was added to the acid filtrate, the solution brought to boiling, and a saturated solution of sodium bisulphite added. The precipitate was separated by filtration, washed, suspended in hot water, and decomposed with hydrogen sulphide. The purine bases separate from the filtrate on concentration. Hypoxanthine, if the only one present, separates only on concentration to a small volume, as it is somewhat soluble in water. Its solubility is much influenced by the presence of impurities which accompany it in this procedure, and statements regarding its solubility by different authorities are very conflicting.

The free base obtained in this way is usually a microcrystalline powder. It forms a hydrochloride of characteristic crystalline appearance, prisms, usually in clusters. It is dissociated by solution in water into the free base and hydrochloric acid.

Hypoxanthine does not give the xanthine reaction with nitric acid and alkalies, but if evaporated with bromine water and nitric acid a residue is left which turns red on heating with alkalies. Like other purine bases it forms a gelatinous precipitate when ammoniacal silver nitrate is added to an ammoniacal solution, and this is dis-

[a] Salomon, Dubois Archiv, pp. 166, 361 (1881); Salomon, Bot. Jahr (1), p. 290 (1880); Schulze, Landw. Jahr., **12**, 912 (1883); Schulze, Landw. Versuch., **46**, 383 (1895); Menozzi, Ber. chem. Ges., **21**, Ref. 619 (1888); Baginsky, Zeit. physiol. Chem., **8**, 395 (1884); Kossel, Zeit. physiol. Chem., **13**, 298 (1889).

solved by boiling nitric acid (sp. gr. 1.1) and separates from this solution immediately on cooling in characteristic miscroscopic needles or plates.

The identity of the compound obtained from the soil was established as a purine base by the method of its separation and its identity as hypoxanthine proved by the appearance and properties of the free base, the crystalline appearance of the hydrochloride, its behavior with bromine water and caustic soda, and the characteristic crystalline appearance of the silver nitrate compound.

Hypoxanthine was discovered first in the spleen and muscles of the heart, and like xanthine has been found to be widely distributed in plants and animals. It has been found in the juice of potatoes, in the juice of sugar beets, and together with xanthine in most plants where that compound has been found.[a]

CONCLUSION.

The methods by means of which the isolation of the substances from the organic matter of soils was accomplished may be briefly shown in the accompanying schematic representation, although for actual working details the text must be consulted.

In the preceding pages and in an earlier bulletin the isolation of 20 organic compounds from soils has been described and while the compounds isolated from any one soil is in most cases but a portion of the soil organic matter, the data obtained are sufficient to warrant certain conclusions regarding this important soil constituent.

In the first place it is evident that the organic matter of soils is made up of a large number of chemical compounds. This might very well be assumed on theoretical grounds and it has been pointed out in previous bulletins [b] that plants, which constitute the greater portion of the material out of which the organic matter of soils is made, contain a vast number of chemical compounds that are added to the soil on the death and decay of the plants. Now, while there seems to be no disposition to question this fact, there has been the assumption on the part of many agriculturists that in some mysterious way this conglomerate of plant compounds added to the soil becomes transformed by decomposition into a single group of closely related bodies, humic acid, etc., and that no matter how varied may be the organic remains, or how diverse the condition of decay, soils vary in organic matter chiefly in amount. That this is not so is sufficiently proven by the facts here established.

Again, not only is the organic matter of soils made up of a large number of compounds, but these compounds represent a number of

[a] Schulze, Landw. Versuch., **28**, 111 (1883); Landw. Jahr., **12**, 912 (1883); Lippmann, Ber. chem. Ges., **29**, 2645 (1896).

[b] Buls. 47 and 53, Bureau of Soils, U. S. Dept. Agr.

Method of separation.					Class.	Compound isolated and identified.
Alkaline extract.	Humus precipitate.	Soluble in alcohol.	Soluble in petroleum ether.	Insoluble in cold alcohol.	Acids	α-Hydroxystearic acid.
				Soluble in cold alcohol.	Acids	Paraffinic acid.
					Fats	Liquid glycerides.
			Insoluble in petroleum ether		Resinous bodies	Resin acids.
						Resin esters.
		Insoluble in alcohol.				
	Acid filtrate.	Shaken out by ether			Acids	Dihydroxystearic acid.
		Precipitated by silver nitrate in neutral solution			Acids	Picoline carboxylic acid.
		Precipitated by silver nitrate in alkaline solution			Pyrimidine derivatives.	Cytosine.
					Hexone bases	Histidine.
						Arginine.
		Precipitated by lead acetate in alkaline solution			Pentosan	Xylan.
		Precipitated by copper sulphate and sodium bisulphite			Purine bases	Xanthine.
						Hypoxanthine.
Hot alcohol extract.	Insoluble in cold alcohol, saponify.	Soluble in petroleum ether.	Insoluble in cold alcohol		Hydrocarbons	Hentriacontane.
			Soluble in cold alcohol		Cholesterol compounds.	Phytosterol.
						Agrosterol.
		Insoluble in petroleum ether			Acids	Agroceric acid.
						Lignoceric acid.
	Soluble in cold alcohol.	Water soluble.				
		Water insoluble	Ether insoluble.			
			Ether soluble	Ammonium carbonate, soluble.	Resinous bodies	Resin acid I.
				Sodium carbonate, soluble.	Resinous bodies	Resin acid II.
				Sodium hydrate, soluble.	Resinous bodies	Resin acid III.
				Undissolved	Resinous bodies	Resin ester.

distinct chemical groups or classes. In the 20 compounds isolated and described, there are 9 classes of chemical compounds represented and when consideration is given the fact that there are many indications of the presence of compounds of other classes where as yet there has been no compound isolated, the conclusion is warranted that the types of compounds in the soil organic matter are quite as varied as in plants or animals. While it is true that the tendency in the decay of organic matter is toward simpler compounds and ultimately to a few simple compounds or the elements, the material known as soil organic matter is in the transition stage from the complex compounds of living organisms to the simple ultimate products. When the organic matter has decayed to the stage of such simple compounds as ammonia, nitric acid, free nitrogen or carbon dioxide, it is no longer organic matter and much of it may escape from the soil altogether.

The fact that the organic matter of the soil is made up of a large number of compounds representing many types throws some light on the difficulties that the early workers on this subject encountered and explains why such diverse results were obtained. This may best be illustrated by the chart below:

Extract soil with alkali.

Alkaline solution, acidify.		Insoluble.
Acid filtrate.	Precipitate.	
So-called *crenic* and *apocrenic acids.*	So-called *humic* and *ulmic acids.*	So-called *humin* and *ulmin.* Identity of components unknown.
Compounds isolated from this filtrate and described in this bulletin:	Compounds isolated from this precipitate and described in this bulletin:	
Dihydroxystearic acid.[a]	*Resin acids.*	
Picoline carboxylic acids.[a]	*Resin esters.*	
Xanthine.	*Glycerides.*	
Hypoxanthine.	*Paraffinic acid.*	
Cytosine.	*Lignoceric acid.*	
Histidine.	*Agroceric acid.*[a]	
Arginine.	*Agrosterol.*[a]	
Pentosan.	*Phytosterol.*	

Probably no one soil contains all the compounds mentioned in the chart, but when two or more occur together in different soils the proportion is generally quite different, and there are, moreover, in all soils many compounds yet unidentified, so that it is no mystery why Mulder and his coworkers, as well as their modern followers, obtained widely different figures for the elementary composition of what they supposed were single compounds.

The complexity of the organic matter shown to exist makes the interpretation of soil phenomena in the light of its chemical composition more difficult and emphasizes the need of a thorough under-

[a] More fully described in Bulletin 53 of this Bureau.

standing of the nature of this material in handling properly the chemical, physical, and biological problems presented by soils. If the soil organic matter were simple in composition, the problems connected therewith would also be simple and the results obtained easily interpreted. Take for instance a single compound, lignoceric acid, that occurs in many soils in fair amount. It would be a comparatively simple problem to determine in what way this compound affects the physical properties of powdered quartz or other rock when added to it in different proportions, or to determine its effect, if any, on the growth of plants or the bacterial flora of soils; and if it were the only organic compound in a soil, come to some conclusion. But it is only one of many compounds and if the factors were known for each of them separately, the factors are not necessarily additive, and the problems presented would still be far from a solution. This recognition of the complexity in the soil and of its problems is, however, a step in the right direction, for the difficult problems presented could never be solved without a full understanding of this very complexity. In short, the chemical relations between the various ingredients of a soil are so complex and the relation of these to living plants so much more so, that it is impossible to make agriculture a true science. Progress, then, along this line seems to lie in the determination of predominant or indicating factors, much the same basis that the modern practice of medicine is founded on. It is quite possible for some form of organic matter to be the determining factor in some physical or biological condition, favorable or otherwise, but this can not be determined unless the nature of the organic compounds in the soil are known, and the methods for identifying them worked out. Moreover, knowledge of the chemical identity of the organic compounds in soils gives a scientific explanation for some of the methods of practical agriculture and affords a foundation for making recommendations with a certainty otherwise impossible. This is well illustrated in the case of dihydroxystearic acid, a compound known in the laboratory to be easily oxidized. In actual practice it has been found that treatments that will promote oxidation either in the soil itself, as by aeration, cultivation, or drainage, or the application of lime or nitrates which stimulate biological oxidation, whether by roots or otherwise, tend to the destruction of this compound.

Finally, the work reported here shows that while the chemistry of the soil organic matter is complex it is not hopelessly so. As was stated in the introduction, no systematic attempt has as yet been made to account by isolated compounds for all of the organic matter in the soils studied. However, in one soil this is so nearly approached that it is offered as an illustration of what has been accomplished in

this direction. This is represented in the chart below. A soil containing 0.955 per cent organic carbon was extracted in quantities of 10 grams with 2 per cent sodium hydroxide solution until no more organic matter was extracted, the soil washed free of alkali, and the carbon determined in the residue. The solution was treated as indicated. Some of the figures were, of course, obtained by difference, and all of the compounds mentioned did not occur in this soil, but are named in the place where they would be found if present.

Soil containing 0.955 per cent organic carbon.
Extracted with 2 per cent sodium hydroxide.

Insoluble.
Represents 24.1 per cent of the total carbon.
Identity of components unknown.

Alkaline solution.
Contains 75.9 per cent of the total carbon.

The alkaline solution, acidified and filtered.

Precipitate.
Represents 36.9 per cent of the total carbon.

Acid filtrate.
Contains 39.0 per cent of the total carbon.
Would contain, if present:
Dihydroxystearic acid.
Xanthine.
Hypoxanthine.
Cytosine.
Histidine.
Arginine.
Pentosan.

Extracted with boiling alcohol.

Insoluble.
Represents 15.7 per cent of the total carbon.
Identity of components unknown.

Alcohol solution.
Contains 21.2 per cent of total carbon.

Extracted with petroleum ether.

Insoluble.
Represents 19.1 per cent the total carbon.
Would contain, if present:
Resin acids.
Resin esters.

Petroleum ether solution.
Contains 2.1 per cent of the total carbon.
Would contain, if present:
Hydroxystearic acid.
Paraffinic acid.
Lignoceric acid.
Glycerides.
Agrosterol.
Phytosterol.

The two fractions with unknown components making 39.8 per cent of the total carbon have been but slightly investigated. It has been found, however, that the portion insoluble in alcohol on again dissolving in alkali and repeating the separation with alcohol is much reduced in quantity, a portion being rendered soluble in alcohol and transferred to the portion insoluble in petroleum ether. In the soil examined the portion soluble in petroleum ether, 2.1 per cent of the total carbon was wholly accounted for in isolated compounds, as was the portion insoluble in petroleum ether and representing 19.1 per cent of the total carbon. The portion in the acid filtrate in the case of most soils represents 40 to 50 per cent of the total carbon and contains a considerable quantity of unidentified material in addition to the large number already isolated and identified.

SUMMARY.

In this bulletin is given some definite knowledge of the nature of the organic compounds in the soil, and methods are described by which a number of organic compounds have been isolated from soils.

The compounds obtained have been described and the manner in which they have been identified pointed out, the possible sources of the compounds suggested, and their relation to other compounds stated.

In a previous bulletin the isolation of four organic compounds from soils was described: Dihydroxystearic acid, $C_{18}H_{36}O_4$; picoline carboxylic acid, $C_7H_7O_2N$; agroceric acid, $C_{21}H_{42}O_3$; and agrosterol, $C_{26}H_{44}O.H_2O$. The compounds in this bulletin, 16 in number, are shown to belong to eight different classes of chemical compounds, some containing carbon and hydrogen only, some containing carbon, hydrogen, and oxygen, and some containing carbon, hydrogen, oxygen, and nitrogen. Paraffin hydrocarbons, acids, alcohols, esters, carbohydrates, hexone bases, pyrimidine derivatives, and purine bases are represented. The list of isolated and identified compounds comprises: Hentriacontane, $C_{31}H_{64}$; monohydroxystearic acid, $C_{18}H_{36}O_3$; paraffinic acid, $C_{24}H_{48}O_2$; lignoceric acid, $C_{24}H_{48}O_2$; phytosterol, $C_{26}H_{44}O.H_2O$; pentosan, $C_5H_8O_4$; histidine, $C_6H_9O_2N_3$; arginine, $C_6H_{14}O_2N_4$; cytosine, $C_4H_5ON_3.H_2O$; xanthine, $C_5H_4O_2N_4$; hypoxanthine, $C_5H_4ON_4$; fatty glycerides and several resin acids and esters.

The conclusion is reached that while the work here reported shows the complex character of the organic matter of soils, this complexity is not so great that the chemical nature of all of the organic matter of soils can not be determined by modern methods of research.

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